

Ephemeral and persistent pseudobulbs differ in their influence on nutrient relations of Guam *Bulbophyllum* plants

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ABSTRACT

Understanding the mechanisms that epiphytic orchids use to sustain plant nutrition may benefit horticulturists and conservationists. Nutrient concentrations of green and senesced leaves and pseudobulbs of *Bulbophyllum guamense* and *Bulbophyllum raulersoniae* were quantified to more fully understand the differences between ephemeral and persistent pseudobulbs. Nutrient content of ephemeral, senescing *B. guamense* pseudobulbs subtending senescing leaves declined in synchrony with leaf senescence. Contrarily, nutrient content of persistent *B. raulersoniae* pseudobulbs subtending senescing leaves increased during leaf senescence. Persistent green leafless *B. raulersoniae* pseudobulbs contained nutrients in quantities similar to pseudobulbs subtending young, green leaves. Nutrient resorption efficiency of pseudobulbs exceeded that of leaves. When *B. raulersoniae* leafless pseudobulbs ultimately senesced, the relative decline in nutrient content exceeded that of senescent *B. guamense* pseudobulbs. The results indicated the persistent pseudobulb behaviors of *B. raulersoniae* may enable buffering of residence times of nutrient reserves to support sink activities of the plant.

Keywords: *Bulbophyllum guamense*, *Bulbophyllum raulersoniae*, Mariana Islands, resorption

1. INTRODUCTION

Epiphytic plants have developed organ modifications to survive in nutrient deficient conditions (Zotz and Hietz, 2001; Zhang et al., 2018). Specialized roots and fleshy storage organs are two of those adaptations (Zotz and Winkler, 2013). Pseudobulbs are common stem modifications exploited by some orchids for storage of resources (Ng and Hew, 2000). Among these resources are water, carbohydrates, secondary metabolites and nutrients (Ng and Hew, 2000; Yang et al., 2016; Sharifi-Rad, 2022). The pseudobulb and leaf act as a module in a clonal plant, with all resources moving between the rhizome and leaf being translocated through the pseudobulb.

Bulbophyllum guamense Ames (Raulerson and Rinehart, 1992) and *Bulbophyllum raulersoniae* Deloso et al., (2022) are two sympatric epiphytic orchid species on the island of Guam (Figure 1A, B). Although the

inflorescence traits are distinctive, the leaf, pseudobulb and rhizome structures of the two species are similar in appearance. Both species produce one leaf per pseudobulb. Gestalt appearance of the entire plant is highly contrasting for the two species, as pseudobulbs are ephemeral for *B. guamense* and senesce in concert with leaves, but pseudobulbs are persistent for *B. raulersoniae* after leaf senescence (Deloso et al., 2022).

No investigations have been conducted to date addressing the contrast of pseudobulb behavior for the two orchid species. Therefore, two investigations were undertaken to determine leaf and pseudobulb nutrient contents of both species and define how persistent pseudobulbs of *B. raulersoniae* influenced nutrient relations. First, I hypothesized that simultaneous senescence of *B. guamense* leaves and pseudobulbs would cause the direction of change in nutrient content to be similar for the two senescing organs. Second, I hypothesized the delayed senescence of *B. raulersoniae* pseudobulbs would cause pseudobulb nutrient content to increase during leaf senescence as a result of leaf nutrient resorption.

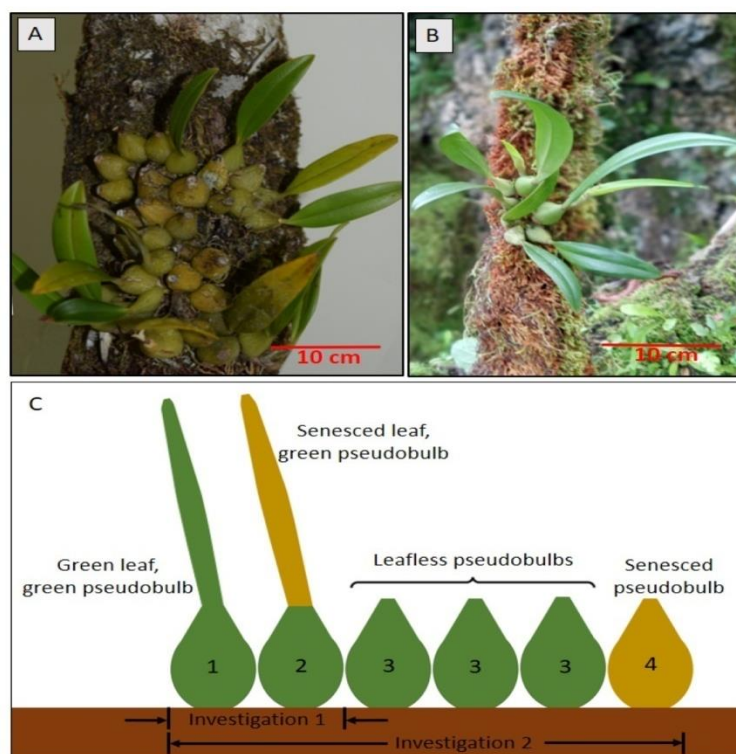


Figure 1 (A) General appearance of *Bulbophyllum raulersoniae* plant with numerous leafless pseudobulbs. (B) General appearance of *Bulbophyllum guamense* plant with no leafless pseudobulbs. (C) Graphical depiction of four types of leaf/pseudobulb modules that were sampled.

2. MATERIALS AND METHODS

Bulbophyllum raulersoniae and *B. guamense* plants were located within a single habitat in southern Guam with karst soils. The habitat was visited in July 2015 to locate suitable host trees with both orchid species on stems with the same orientation. The surveyed area was centered at 13.340855 °N, 144.668139 °E and spanned 0.8 km latitudinally and 0.6 km longitudinally.

Investigation 1

Four *Elaeocarpus joga* Merr. trees and two *Guettarda speciosa* L. trees were selected as host trees for this investigation. All *Bulbophyllum* plants were located on stems that were oriented less than 10° above or below the horizontal to ensure stemflow was similar for all replications. Moreover, each *Bulbophyllum* plant was positioned at least 100 cm away from an adjacent plant to ensure one plant did not influence the nutrition of a second plant.

Leaf and pseudobulb selection followed established protocols for determining nutrient resorption efficiency (Aerts, 1996; Killingbeck, 1996). Plants with flowers or fruits were not included to ensure the reproductive organs did not influence the results. The youngest fully expanded leaf was collected to represent green leaves and the subtending pseudobulb was also collected. Yellow leaves that had not abscised were collected to represent senesced leaves. The pseudobulb subtending the senesced leaves was

collected at the same time. For *B. guamense* these pseudobulbs were also senescent, for *B. raulersoniae* the pseudobulbs were green. The field sampling was conducted during several days in August 2015.

The samples were processed as previously described (Marler, 2018). Leaf and pseudobulb tissues were dried at 75 °C and milled to pass through a 20-mesh screen. Total carbon (C) and nitrogen (N) were determined by dry combustion (FLASH EA1112 CHN Analyzer, Thermo Fisher, Waltham and Mass). Samples were also digested by a microwave system with nitric acid and peroxide, then phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were quantified by inductively coupled plasma optical emission spectroscopy (Spectro Genesis; SPECTRO Analytical Instruments, Kleve, Germany).

Each host tree was considered one replication and there were six replications. The response variables met parametric requirements. The data were subjected to a species × organ age factorial ANOVA. The leaf and pseudobulb data were analyzed separately. Means separation was by Tukey's HSD. Resorption efficiency was calculated for N, P and K as $((\text{young} - \text{old}) / \text{young}) \times 100$ for leaves and for the pseudobulbs that subtended the green and senesced leaves. All statistical procedures were conducted with SAS (SAS Institute, Cary, NC, U.S.A.).

Investigation 2

Bulbophyllum raulersoniae plants were more prevalent on orthotropic host stems. Therefore, three *Areca catechu* L. and three *Pandanus tectorius* Parkinson ex Zucc. were used for this investigation. The mean stem angle above the horizontal ranged from 82° to 89°. The habitat was surveyed in September 2019 to locate the appropriate plants for this study by identifying plants with numerous leafless green pseudobulbs (Figure 1A). Plants on stems that contained a different epiphyte above the potential experimental plant were avoided.

Sample collections on September 22, 2019 were guided by prescribed pseudobulb categories (Figure 1C). Leaves and pseudobulbs were harvested in accordance with the methods in Investigation 1 to provide Categories 1 and 2. Additionally, several green pseudobulbs that persisted after leaf abscission were harvested as Category 3. Senescent pseudobulbs were harvested as Category 4.

The tissue samples were analyzed for nutrients as described for Investigation 1. Each host tree was considered one replication and there were six replications. Differences in nutrient content between green and senesced leaves were determined by *t*-test. The pseudobulb data were subjected to one-way ANOVA of four pseudobulb age categories. Resorption efficiencies of N, P and K were calculated for leaves as previously described. Resorption efficiency of pseudobulbs was calculated from Categories 1 (young) and 4 (old).

3. RESULTS

Investigation 1

Every leaf nutrient that was measured exhibited differences between the two species, with the exception of Mn (Table 1). Similarly, every nutrient except B, C and Cu exhibited differences between the two leaf age categories. The extent of interaction between species × age was variable among the nutrients. The macronutrients C and N and the micronutrients B and Cu were not influenced by the interaction, but the remainder of the nutrients exhibited significant interactions (Table 1).

Leaf Ca was greater in *B. raulersoniae* leaves than in *B. guamense* leaves and did not change during leaf senescence (Table 2). In contrast, leaf Ca increased as *B. guamense* leaves senesced. Leaf Mg decreased in a similar manner for the two species as leaves senesced, but Mg was less in *B. raulersoniae* leaves. Leaf P and K were also less in *B. raulersoniae* leaves, but P and K in *B. guamense* leaves decreased to a greater degree during senescence. Green leaf Fe and Zn were similar for the two species, but Fe and Zn increased more during senescence for *B. raulersoniae*. Green leaf Mn was also similar for the two species and decreased during senescence to a greater degree for *B. guamense*.

Table 1 Results from 2 species × 2 organ age factorial ANOVA for tissue content of essential nutrients in *Bulbophyllum* leaves and pseudobulbs.

Response variable	Species <i>F</i> _{1,20}	Species <i>P</i>	Age <i>F</i> _{1,20}	Age <i>P</i>	S×A <i>F</i> _{1,20}	S×A <i>P</i>
Leaves						
Calcium	104.66	<0.001	8.92	0.007	8.18	0.010
Carbon	54.80	<0.001	1.88	0.186	0.98	0.334

Magnesium	313.55	<0.001	723.71	<0.001	35.113	<0.001
Nitrogen	139.41	<0.001	267.12	<0.001	1.54	0.229
Phosphorus	30.99	<0.001	93.41	<0.001	15.92	<0.001
Potassium	66.39	<0.001	425.50	<0.001	136.83	<0.001
Boron	64.58	<0.001	1.50	0.235	0.668	0.423
Copper	6.40	0.020	0.42	0.534	1.63	0.220
Iron	134.54	<0.001	139.74	<0.001	178.96	<0.001
Manganese	4.05	0.058	126.73	<0.001	7.39	0.013
Zinc	15.43	<0.001	22.61	<0.001	65.00	<0.001
Pseudobulbs						
Calcium	227.48	<0.001	1.66	0.213	1.83	0.191
Carbon	212.20	<0.001	0.69	0.417	0.08	0.776
Magnesium	170.31	<0.001	123.50	<0.001	124.65	<0.001
Nitrogen	2634.81	<0.001	25.27	<0.001	427.50	<0.001
Phosphorus	11.10	0.003	54.28	<0.001	131.80	<0.001
Potassium	0.59	0.451	38.14	<0.001	98.48	<0.001
Boron	33.48	<0.001	1.25	0.276	5.89	0.025
Copper	0.91	0.352	0	1.000	0	1.000
Iron	19.72	<0.001	1.13	0.299	0.03	0.856
Manganese	836.69	<0.001	20.35	<0.001	96.41	<0.001
Zinc	19.30	<0.001	0.26	0.615	0.51	0.483

Table 2 Leaf nutrient content of *Bulbophyllum guamense* and *Bulbophyllum raulersoniae* plants as influenced by organ age. Mean \pm SE, n=6.

Element	<i>B. guamense</i> green	<i>B. guamense</i> senesced	<i>B. raulersoniae</i> green	<i>B. raulersoniae</i> senesced
Leaves				
Calcium (mg·g ⁻¹)	7.8 \pm 0.3c ¹	9.5 \pm 0.2b	11.1 \pm 0.3a	11.7 \pm 0.3a
Magnesium (mg·g ⁻¹)	6.0 \pm 0.1a	3.7 \pm 0.1c	4.3 \pm 0.1b	2.8 \pm 0.1d
Phosphorus (mg·g ⁻¹)	1.0 \pm 0.1a	0.6 \pm 0.1b	0.8 \pm 0.1b	0.6 \pm 0.1b
Potassium (mg·g ⁻¹)	9.7 \pm 0.4a	3.0 \pm 0.3c	5.6 \pm 0.3b	3.7 \pm 0.2c
Iron (μg·g ⁻¹)	57.9 \pm 2.0c	84.5 \pm 2.1b	54.0 \pm 1.7c	110.9 \pm 3.1a
Manganese (μg·g ⁻¹)	63.4 \pm 5.6a	20.7 \pm 1.8b	61.4 \pm 4.4a	35.2 \pm 1.8b
Zinc (μg·g ⁻¹)	15.8 \pm 0.8c	18.1 \pm 0.8b	14.4 \pm 0.5c	24.8 \pm 1.2a
Pseudobulbs				
Magnesium (mg·g ⁻¹)	5.4 \pm 0.3a ¹	2.5 \pm 0.2b	2.3 \pm 0.1b	2.3 \pm 0.2b
Nitrogen (mg·g ⁻¹)	1.7 \pm 0.1c	0.3 \pm 0.1d	3.4 \pm 0.3b	4.2 \pm 0.3a
Phosphorus (mg·g ⁻¹)	1.4 \pm 0.1a	0.6 \pm 0.1c	0.7 \pm 0.1bc	0.9 \pm 0.1b
Potassium (mg·g ⁻¹)	13.6 \pm 0.7a	7.2 \pm 0.4d	9.3 \pm 0.3c	10.8 \pm 0.3b
Boron (μg·g ⁻¹)	14.7 \pm 1.3b	11.9 \pm 0.6c	18.0 \pm 0.6a	19.2 \pm 0.8a
Manganese (μg·g ⁻¹)	7.5 \pm 0.9c	4.3 \pm 0.3d	19.5 \pm 0.8b	28.5 \pm 1.1a

¹ Mean with same letter within each row are not different according to Tukey's HSD.

The four nutrients that did not exhibit a species \times age interaction for leaf tissues were different between the species (Table 1). Leaf C was greater for *B. guamense* (284.7 \pm 2.8 mg·g⁻¹) than for *B. raulersoniae* (463.8 \pm 2.6 mg·g⁻¹). Similarly, leaf N was greater for *B. guamense* (6.4 \pm 0.5 mg·g⁻¹) than for *B. raulersoniae* (4.5 \pm 0.4 mg·g⁻¹). In contrast, leaf B was greater for *B. raulersoniae* (36.6 \pm 1.2 μg·g⁻¹) than for *B. guamense* (26.8 \pm 1.1 μg·g⁻¹) and leaf Cu was greater for *B. raulersoniae* (2.2 \pm 0.2 μg·g⁻¹) than for *B. guamense* (1.6 \pm 0.2 μg·g⁻¹). Leaf B, C and Cu did not exhibit a significant leaf age effect. In contrast, leaf N was greater in green (6.8 \pm 0.4 mg·g⁻¹) than in senesced leaves (4.1 \pm 0.3 mg·g⁻¹).

Nitrogen resorption efficiency (NRE) of *B. raulersoniae* was 45% and NRE of *B. guamense* 37%. In contrast, potassium resorption efficiency (KRE) of *B. raulersoniae* was 33% and KRE of *B. guamense* was 68%. Phosphorus resorption efficiency (PRE) of *B. raulersoniae* was 21% and PRE of *B. guamense* was 38%. The relationships of pseudobulb nutrients among the species and age factors were not similar to those of leaves (Table 1).

Every nutrient that was measured exhibited differences between the two species, with the exception of Cu and K. Pseudobulb Mg, N, P, K and Mn also exhibited differences between the two age categories. The interaction of species \times age was variable among the nutrients. Pseudobulb Mg, N, P, K, B and Mn exhibited significant interactions and the remainder of the nutrients did not exhibit a significant interaction (Table 1).

The differences among the four species \times age categories were greater for pseudobulbs than for leaves (Table 2). Pseudobulb Mg was greatest for green *B. guamense* leaves and Mg did not differ among the other three categories. Tissue N increased as pseudobulbs aged for *B. raulersoniae*, but greatly decreased as *B. guamense* pseudobulbs senesced. Tissue P was greatest for green *B. guamense* pseudobulbs, intermediate for senesced *B. raulersoniae* pseudobulbs and least for the other two interaction categories. Pseudobulb B was greatest for the two *B. raulersoniae* categories, intermediate for the green *B. guamense* and least for the senesced *B. guamense*.

Pseudobulb Ca, C, Fe and Zn did not exhibit a species \times age interaction, but did exhibit a significant species effect (Table 1). Pseudobulb Ca was greater for *B. raulersoniae* ($8.8 \pm 0.2 \text{ mg}\cdot\text{g}^{-1}$) than for *B. guamense* ($5.4 \pm 0.2 \text{ mg}\cdot\text{g}^{-1}$). Pseudobulb C was greater for *B. raulersoniae* ($452.7 \pm 0.1.9 \text{ mg}\cdot\text{g}^{-1}$) than for *B. guamense* ($421.9 \pm 1.8 \text{ mg}\cdot\text{g}^{-1}$). Similarly, pseudobulb Zn was greater for *B. raulersoniae* ($18.0 \pm 0.7 \text{ }\mu\text{g}\cdot\text{g}^{-1}$) than for *B. guamense* ($14.6 \pm 0.4 \text{ }\mu\text{g}\cdot\text{g}^{-1}$). In contrast, pseudobulb Fe was greater for *B. guamense* ($67.8 \pm 1.9 \text{ }\mu\text{g}\cdot\text{g}^{-1}$) than for *B. raulersoniae* ($57.7 \pm 1.2 \text{ }\mu\text{g}\cdot\text{g}^{-1}$). Pseudobulb C, Ca, Fe and Zn did not exhibit a significant age effect (Table 3). Pseudobulb Cu was the only nutrient that did not exhibit significance for any of the factors. The overall Cu mean was $1.9 \pm 0.3 \text{ }\mu\text{g}\cdot\text{g}^{-1}$.

Pseudobulb nutrient resorption efficiencies were contrasting for the two *Bulbophyllum* species. For *B. guamense*, all three macronutrients declined as the pseudobulbs senesced in synchrony with leaf senescence, so NRE was 80%, KRE was 46% and PRE was 59%. In contrast, these macronutrients increased for the green *B. raulersoniae* pseudobulbs that subtended the senescent leaves (Category 2 in Figure 1C). Therefore, the resorption efficiencies were -27% for NRE and PRE and -15% for KRE.

Investigation 2

The macronutrient content of green and senescent *B. raulersoniae* leaves was similar to that of Investigation 1 (Figure 2A). Green leaf N was 80% greater than senescent leaf N ($t=27.59$, $P<0.001$), green leaf P was 31% greater than senescent leaf P ($t=5.55$, $P<0.001$), green leaf K was 55% greater than senescent leaf K ($t=44.08$, $P<0.001$) and green leaf Mg was 67% greater than senescent leaf Mg ($t=10.29$, $P<0.001$). Leaf C did not differ between the leaf age categories ($t=0.05$, $P=0.480$) and the mean was $465.9 \pm 1.9 \text{ mg}\cdot\text{g}^{-1}$. Leaf Ca also did not differ between the leaf age categories ($t=0.40$, $P=0.348$) and the mean was $11.4 \pm 0.3 \text{ mg}\cdot\text{g}^{-1}$.

The micronutrient content of green and senescent *B. raulersoniae* leaves was also similar to that of Investigation 1 (Figure 2B). Senescent leaf Fe was 102% greater than green leaf Fe ($t=12.48$, $P<0.001$), green leaf Mn was 97% greater than senescent leaf Mn ($t=18.99$, $P<0.001$) and senescent leaf Zn was 84% greater than green leaf Zn ($t=9.40$, $P<0.001$). Leaf B did not differ between the leaf age categories ($t=0.412$, $P=0.344$) and the mean was $37.1 \pm 0.9 \text{ }\mu\text{g}\cdot\text{g}^{-1}$. Leaf Cu also did not differ between the leaf age categories ($t<0.001$, $P=0.500$) and the mean was $2.1 \pm 0.2 \text{ }\mu\text{g}\cdot\text{g}^{-1}$.

Every macronutrient was influenced by *B. raulersoniae* pseudobulb age, with the senescent pseudobulbs exhibiting the least content (Table 3). The Ca content of senescent pseudobulbs was 18% below that of the other three age categories ($F_{3, 20}=816$, $P<0.001$) and the C content of senescent pseudobulbs was 10% below that of the other three age categories ($F_{3, 20}=248.2$, $P<0.001$). These two macronutrients were not different among the other pseudobulb age categories. Pseudobulb K, Mg, N and P content behaved similarly among the age categories. The initial content of pseudobulbs that sub-tended green leaves (Category 1) increased during leaf senescence (Category 2) as nutrient resorption from leaves progressed. Thereafter, the macronutrient content of green leafless pseudobulbs (Category 3) declined to levels that were not different from the initial pseudobulbs that subtended green leaves. Finally, these macronutrients declined during ultimate senescence of the pseudobulbs (Category 4). These differences were highly significant for Mg ($F_{3, 20}=90.5$, $P<0.001$), N ($F_{3, 20}=650.2$, $P<0.001$), P ($F_{3, 20}=113.6$, $P<0.001$) and K ($F_{3, 20}=151.7$, $P<0.001$).

Some of the micronutrients also contrasted among *B. raulersoniae* pseudobulb age categories (Table 3). Pseudobulb Fe content of senescent pseudobulbs was 18% greater than the other three age categories ($F_{3, 20}=8.9$, $P<0.001$). Similarly, pseudobulb Zn of senescent pseudobulbs was 25% greater than the other three age categories ($F_{3, 20}=5.5$, $P=0.007$). Pseudobulb Mn exhibited a pattern among the age categories that was similar to the macronutrients, with an increase in content as leaves senesced, then a decline as the leafless pseudobulbs continued to age, followed by the senescent pseudobulbs exhibiting the least amount of Mn ($F_{3, 20}=26.6$, $P<0.001$). Pseudobulb B ($F_{3, 20}=1.1$, $P=0.406$) and Cu ($F_{3, 20}=0.1$, $P=0.972$) were not influenced by pseudobulb age.

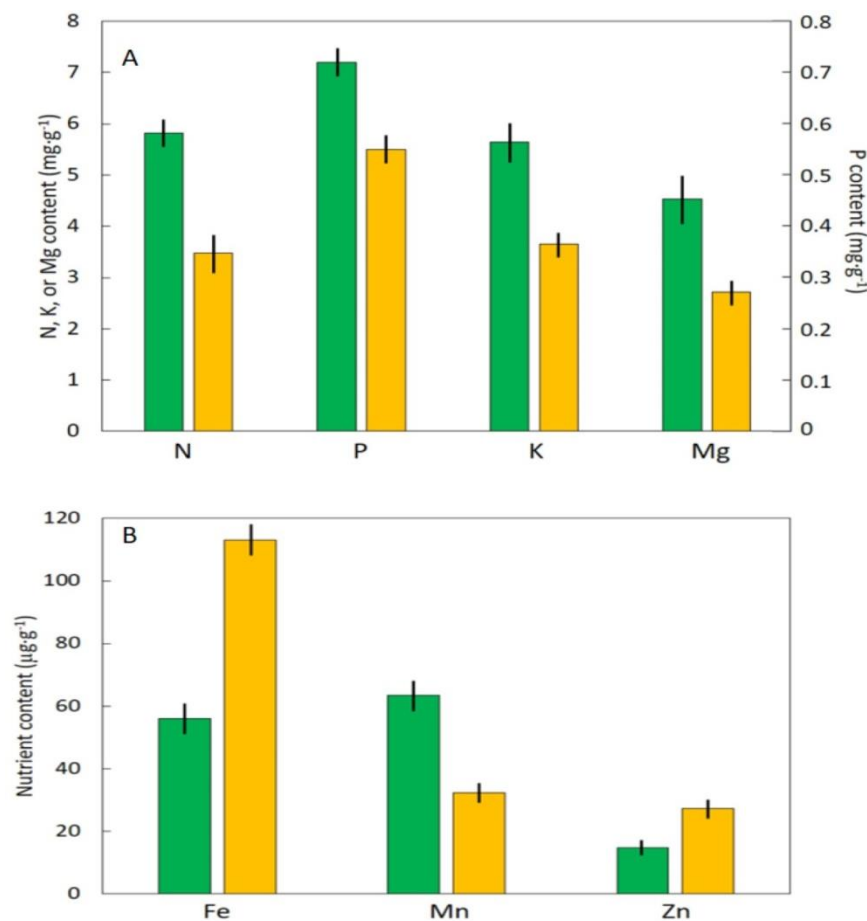


Figure 2 Macronutrient (A) and micronutrient (B) content of *Bulbophyllum raulersoniae* mature green (green) and senescent (yellow) leaves. Mean \pm SE, $n=6$.

The nutrient resorption efficiencies for N, P and K differed between *B. raulersoniae* leaves and pseudobulbs. NRE was 72% for pseudobulbs and 44% for leaves ($t=17.734$, $P<0.001$). KRE was 47% for pseudobulbs and 35% for leaves ($t=3.03$, $P=0.006$). PRE was 41% for pseudobulbs and 24% for leaves ($t=2.41$, $P=0.018$).

Table 3 Nutrient content of *Bulbophyllum raulersoniae* pseudobulbs as influenced by age, Category 1 = green pseudobulb subtending green leaf; Category 2 = green pseudobulb subtending senescent leaf; Category 3 = persisting green pseudobulb with no leaf; Category 4 = senescent pseudobulb. Mean \pm SE, $n=6$.

Element	Category 1	Category 2	Category 3	Category 4
Calcium (mg·g ⁻¹)	8.9 \pm 0.3a ¹	9.0 \pm 0.2a	9.0 \pm 0.2a	7.3 \pm 0.2b
Carbon (mg·g ⁻¹)	453.7 \pm 3.7a	452.7 \pm 2.8a	453.5 \pm 3.3a	406.6 \pm 2.9b
Magnesium (mg·g ⁻¹)	2.3 \pm 0.1b	3.2 \pm 0.2a	2.4 \pm 0.1b	1.2 \pm 0.1c
Nitrogen (mg·g ⁻¹)	3.4 \pm 0.3b	4.3 \pm 0.3a	3.5 \pm 0.2b	1.0 \pm 0.1c
Phosphorus (mg·g ⁻¹)	0.8 \pm 0.1b	1.0 \pm 0.1a	0.8 \pm 0.1b	0.5 \pm 0.1c
Potassium (mg·g ⁻¹)	9.4 \pm 0.4b	11.1 \pm 0.3a	9.4 \pm 0.3b	5.4 \pm 0.2c
Boron (µg·g ⁻¹)	20.5 \pm 1.2	21.9 \pm 1.6	21.0 \pm 1.7	18.2 \pm 1.8
Copper (µg·g ⁻¹)	2.1 \pm 0.4	2.3 \pm 0.3	2.3 \pm 0.3	2.0 \pm 0.3
Iron (µg·g ⁻¹)	57.9 \pm 2.6b	59.3 \pm 2.3b	58.8 \pm 1.9b	69.5 \pm 2.1a
Manganese (µg·g ⁻¹)	20.5 \pm 1.6b	27.8 \pm 2.3a	22.2 \pm 1.8b	10.5 \pm 0.7c
Zinc (µg·g ⁻¹)	18.5 \pm 0.6b	18.3 \pm 0.9b	19.0 \pm 1.1b	23.2 \pm 1.2a

¹ Mean with same letter within each row are not different according to Tukey's HSD.

4. DISCUSSION

The pseudobulb is one of the consequential adaptations of epiphytic orchids that enable survival. Pseudobulbs serve as a storage organ for resources that may be deployed to other modules in the orchid plant (Ng and Hew, 2000; Yang et al., 2016; Sharifi-Rad, 2022). The simultaneous senescence of Guam's *B. guamense* leaves and pseudobulbs was hypothesized to generate similar directions of change in nutrient content. This prediction was confirmed for the nutrients that declined during leaf senescence. Delayed senescence of *B. raulersoniae* pseudobulbs was hypothesized to cause pseudobulb nutrient content to increase during leaf senescence. This prediction was confirmed for the pseudobulbs that subtended senescent leaves for all leaf nutrients which were resorbed. The nutrients within the ultimate senescent pseudobulbs exhibited reductions in nutrient content that exceeded that of senescent leaves.

The numerous leafless *B. raulersoniae* pseudobulbs increased the overall plant size compared with *B. guamense*. The nutrients stored within this group of leafless pseudobulbs remains available for deployment, as verified by the ultimate decline in nutrient content in the yellow, senescing pseudobulbs. This considerable storage capacity renders the resources available to support ephemeral sink activities such as construction of new leaves, inflorescences and fruits.

The nutrient resorption results for these orchid species illuminated several interesting traits. The negative resorption efficiency that is calculated when senescent plant organs contain greater quantities of nutrients than green organs have been described as accretion efficiency (Marler, 2021). The orchid leaves exhibited accretion of Ca, Fe and Zn and this behavior has been reported for some other indigenous and endemic Guam plant species (Marler, 2018; Marler, 2021). For *B. raulersoniae*, the sink activity of the entire plant body appeared to be able to absorb more of the nutrient resources from senescing pseudobulbs than was absorbed from *B. guamense* pseudobulbs. For example, senescent *B. raulersoniae* pseudobulbs declined in C and Ca content, but this did not occur for the ephemeral *B. guamense* pseudobulbs or for leaves of either species.

Global efforts in orchid conservation have been inadequate to stop the ongoing threats of orchid extinction events (Phillips et al., 2020). *Bulbophyllum guamense* was listed as Threatened under the United States Endangered Species Act in 2015 (United States Fish and Wildlife Service, 2015). *Bulbophyllum raulersoniae* was undescribed at that time, so the risk of extinction has not been formally assessed. The 2020 report for *B. guamense* recovery efforts (United States Fish and Wildlife Service, 2020) did not include any peer-reviewed journal articles during the first five years of federal listing. This lack of appreciation for conservation research by the decision-makers is one of the greatest ongoing threats to the endangered plant species in the Mariana Islands and has been discussed elsewhere for *Cycas micronesica* K.D. Hill (Marler and Krishnapillai, 2020) and *Serianthes nelsonii* Merr. (Marler et al., 2021).

This study is the second published investigation of endemic orchid nutrient relations for Guam. The relatively low content of leaf nutrients and the nutrient resorption dynamics reported herein corroborated the earlier report (Marler, 2018). These reports illuminate several directions of future research, including efforts to better understand demography, phenology, source-sink dynamics and carbon fixation of pseudobulbs and leaves (Ticktin et al., 2020; Emeterio-Lara, 2021; He et al., 2011; Portela, 2021; Ramírez-Martínez et al., 2021).

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Ethical approval

Bulbophyllum raulersoniae and *B. guamense* plants from southern Guam, were used in the study. The ethical guidelines for plants & plant materials are followed in the study for sample collection & identification.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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